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(54) Abstract Title: **Antimicrobial compounds**

(57) A composition for use as an antimicrobial medicament comprising at least one essential oil and a fungal cell or fungal cell fragment, wherein the essential oil is encapsulated by the fungal cell or cell fragment. The composition is preferably used for the treatment of Staphylococcus infection, particularly methicillin resistant s. aureus (MRSA). Wound dressings comprising the composition are also disclosed. An alternative embodiment provides a composition comprising a biocidal compound and at least one essential oil, and a further embodiment provides a composition comprising a first and a second essential oil.

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ANTIMICROBIAL COMPOSITION

The present invention relates to antimicrobial compositions and methods of using the same In particular the present invention relates to antimicrobial compositions and methods for inhibiting microbial development in wounds

Control of infection in wounds is mainly achieved by fungicides, bactericides and/or antibiotics. However, these synthetic chemicals can be toxic in high concentrations.

Furthermore, the development of resistance to these biocides has been observed in some strains of microorganisms Consequently, many fungicides and bactericides are being phased out by regulating agencies

Despite major advances in wound management, infection still remains an important factor in wound healing For instance, in burns approximately 75% of deaths are due to complications with sepsis from wound infection (1) Among other adverse effects, infection delays healing, contributes to graft failure and can increase the depth of a burn. Approximately 30% of burn wounds become colonized with *Staphylococcus aureus* (2) and outbreaks of methicillin-resistant *S. aureus* (MRSA) have created major problems for burn units and intensive care units in terms of cross infection and rehabilitation of the patient due to imposed barrier nursing (3) Some MRSA strains, such as epidemic MRSA (EMRSA) have the ability to spread rapidly among patients and the dominant clonal EMRSA types 15 and 16 are problematic in the UK (4,5).

Staphylococci are an example of common bacteria that live on the skin and mucous membranes (e.g in the nose) of humans About 15-40 per cent of healthy humans are carriers of *S. aureus*, that is, they have the bacteria on their skin without any active infection or disease (colonisation) *S aureus* is the most pathogenic species of the Genus as they can cause potentially fatal diseases and currently major concern focuses around their increasing resistance to antibiotics In the USA and the UK 90% of *S. aureus* isolates are resistant to penicillin G and incidence of methicillin resistance (MRSA) is rising exponentially

Vancomycin is one of the few effective systemic antibiotics available for treatment, however increased inhibitory concentrations (intermediate resistance) has been reported (Vancomycin intermediate *Staphylococcus aureus* VISA)and there is major concern that total antibiotic resistant strains may emerge in the immediate future (6). However, because of its toxicity and the threat of resistance its use is controlled.

To date topical anti-microbial therapy is the single most important component of wound care to prevent infection (7) and in hospitalised burns patients, Flamazine ™ is by far the most frequently used topical prophylactic agent (8) but does not always penetrate into the wound (9) and cannot be used to eradicate carriage from the patient or the environment. Thus means of preventing infection, reducing colonisation of the patient by microorganisms and thus reducing the need for administered antibiotics is needed.

It is an object of the present invention to alleviate or overcome one or more of the problems associated with the prior art and/or to provide an improved antimicrobial composition. It is a further object of the invention to provide an improved method for inhibiting or preventing microbial development in wounds or other lesions.

In accordance with a first aspect of the present invention there is provided a composition for use as a medicament, which composition comprises at least one essential oil and a fungal cell or fungal cell fragment wherein molecules of the at least one essential oil are encapsulated or partially encapsulated by the fungal cell or fungal cell fragment.

In accordance with a further aspect of the present invention, there is provided a composition comprising at least one essential oil and at least one biocidaland/or antibiotic compound.

The biocidal compound may be a fungicide and/or a bactericide The biocidal compound may be selected from phenols and cresols, acids and esters, alkalis, chlorine release agents, iodine compounds, quaternary ammonium compounds, biguanides, diamidines, aldehydes, alcohols, heavy

metal derivatives, vapour phase disinfectants, sulphates and nitrites, for example Preferably, the biocidal compound is a bactericide More preferably, the bactericide is triclosan (obtainable from Cambiochem California, USA of EMD Biosciences Inc , an affiliate of Merck, Germany). Other preferred biocides include chlorhexidine, povidone iodine and/or silver sulphadiazine, for example.

Preferred antibiotics include mupirocin, fucidin and gentamicin, for example.

In accordance with a further aspect of the present invention, there is provided a composition comprising an essential oil, a biocidal and/or antibiotic compound and a fungal cell or fungal cell fragment, wherein molecules of at least one of the essential oil or biocidal/antibiotic compound are encapsulated or partially encapsulated by the fungal cell or fungal cell fragment.

In accordance with a further aspect of the present invention, there is provided a composition comprising two or more essential oils and a fungal cell or fungal cell fragment wherein molecules of at least one essential oil is encapsulated or partially encapsulated by the fungal cell or fungal cell fragment.

In accordance with a further aspect of the present invention, there is provided a therapeutic formulation comprising a composition as described hereinabove. The formulation may comprise one or more excipients

In accordance with a further aspect of the present invention there is provided the use of a composition for the manufacture of a medicament for the treatment or prophylaxis of microbial infection, the composition comprising at least one essential oil and a fungal cell or fungal cell fragment, wherein molecules of the essential oil are encapsulated or partially encapsulated by the fungal cell or fungal cell fragment.

Preferably, the composition is for the treatment or prophylaxis of Staphylococcus infection. Strains of staphylococcus include S *aureus*, *S epidermidis*, *S. saprophyticus*, *S. haemolyticus*, Methicillin

sensitive *S. aureus* (MSSA), Methicillin resistant *S. aureus* (MRSA) and Epidemic methicillin resistant *S. aureus* (EMRSA) More preferably, the composition is for the treatment of MRSA.

In accordance with a further aspect of the present invention there is provided a method of treating or preventing a microbial infection in a subject comprising administering to a subject a composition as described hereinabove.

In accordance with a further aspect of the present invention there is provided a composition comprising:

a) a first essential oil comprising Manuka and a second essential oil comprising one or more essential oil selected from the group comprising Geranium, Lavender, Lemongrass, and Tea tree, or

b) a first essential oil comprising Geranium and a second essential oil comprising one or more essential oil selected from the group comprising Manuka, Lavender, Lemongrass, and Tea tree; or

c) a first essential oil comprising Lemongrass and a second essential oil comprising one or more essential oil selected from the group comprising Geranium, Lavender, Manuka, and Tea tree; or

d) a first essential oil comprising Lavender and a second essential oil comprising one or more essential oil selected from the group comprising Geranium, Manuka, Lemongrass, and Tea tree, or

e) a first essential oil comprising Tea tree and a second essential oil comprising one or more essential oil selected from the group comprising Geranium, Lavender, Lemongrass, and Manuka.

Essential oils are complex mixtures of odorous, steam volatile or extractable organic compounds, which are synthesised by many types of plant Essential oils can be found in various parts of a plant, such as the leaves, stem, flowers, cell organelles, fruit, roots, seeds and bark etc Generally, the principal constituents are aromatic compounds. Each oil may comprise 100-300 compounds

Essential oils most abundant components include one or more Mono-, di- and sesqui- terpenoids (mevalonic acid derived constituents); phenylpropanoids; alkanes (and alkane derivatives, such as alcohols, aldehydes, and carboxylic acids), alkenes, alkynes and derivatives thereof

Essential oils are typically mixtures of organic aromatic and other compounds that are extractable from plant material by methods such as steam distillation, cold pressing, CO_2 extraction or extraction with organic solvents or any other means known to the person skilled in the art.

Essential oils for use in the present invention include but are not limited to extracts from Bay (Pimenta recemosa), Bergamot (*Citrus bergamia*); Cardamom (*Elettaria cardamom*); Cedarwood (*Cedrus deodara* and *Juniperus virginiana*); Cinnamon leaf (*Cinnamomum zellanicum* Ceylon); Clove or clove bud (*Eugenia caryophyllata* Madagascar extra; *Syzygium aromaticum L./Eugenia aromaticum L*);Cumin seed (*Cuminum cyminum*); Eucalyptus (*Eucalyptus globulus & radiata*); Geranium (*Pelargonium graveolens* Madagascar bourbon); Grapefruit (*Citrus paradisi*); Lavender (*Lavendula officinalis* France);Lemongrass (*Cymbopogon citrates*), Manuka (*Leptospermum scoparium*); Marjoram (*Onganum majorana*); Origanum (*Origanum vulgarel Cymbopogon martini*); Palmarosa (*Origanum heracleoticum*), Patchouli (*Pogostemon cablin* E India dark);Peppermint (*Mentha pipenta*); Rosemary (*Rosmarinus officinalis*); Rosewood (*Aniba rosaeodora*); Sage (*Salvia tribola*); Sandalwood (*Aniba rosaeodora*); Savory (*Satureia thymbra*), Tea Tree (*Melaleuca alternifolal/Leptospermum petersonii*); Thyme (*Thymus capitus*). Other essential oils useful in the present invention include Sandal oil, KapurTulsi oil, and Ropan oil

Preferably, compositions according to the present invention comprise one or more essential oils from the group comprising Manuka, Geranium, Lavender, Lemongrass and Tea tree. More preferably, the compositions of the present invention comprise two or more essential oils selected from the group comprising Manuka, Geranium, Lavender, Lemongrass and Tea tree More preferably still, the composition of the present invention comprises one or more of the following combinations of essential oils Manuka + Geranium, Geranium + Lavender, Manuka + Lavender, Geranium + Lemongrass, Manuka + Lemongrass, Geranium + Tea tree, Manuka + Tea tree, Lavender + Lemongrass, Lemongrass + Tea tree and Lavender + Tea tree.

Other common chemical constituents of essential oils are citral (geranial and neral isomers), limonene, linalyl acetate and estragole (methyl chavicol), mono-, sesqui- and di-terpenoids (mevalonic acid-derived constituents); phenylpropanoids (cinnamic acid-derived compounds) and alkane derivatives (alkanes, alkenes, alkynes, alkanols, alkanals, alkanoic acids: mostly acetogenins).

It is understood that the term "essential oil" as used herein includes the naturally occurring extractable plant oils, mixtures thereof, or one or more of the components found in extractable plant oils, whether naturally or artificially synthesized. The term also includes derivatives and analogues of the components found in extractable plant oils.

Preferably, compositions according to the present invention comprise combinations of essential oil and encapsulated essential oil mixtures in ratios of, for example, 50:50, 75:25 and 25 75, or ratios therebetween Preferably, the combinations are based upon the weight of the product

The fragment of fungal cell may comprise a fungal cell wall, such as a ghost cell, or a part thereof wherein said part is capable of passively retaining the composition Encapsulated compounds are described in WO 00/69440.

The composition preferably contains an essential oil, essential oil mixture and/or a biocide as an active ingredient in an amount effective to inhibit the growth of a pathogen on a surface to which the composition is applied The active ingredient is preferably present in the composition in an amount such that when the composition is applied to a surface, the active ingredient is preferably present in an amount of from about 5 to about 30μg/cm^2 on or over said surface.

The fungal cell or fungal cell fragment is preferably derivable from filamentous fungi, more preferably from fungi having relatively high levels of chitin and/or chitosan. Such species include, but are not limited to, Mucor and Rhizomucor, although other species that are lower in chitin, such as Penicillium, Apergillus and Fusarium may also be suitable The fungal cell or fungal cell fragment may be derived from *Saccharomyces cerevisiae*, such as Bakers yeast, Williams yeast (obtainable from Aventine

Renewable Energy Co., Inc 1300 South 2nd Street, Pekin, Illinois, 61555-00, USA) or DCL blue label yeast obtainable from Lessafre at www.lesaffreyeastcorp.com.

The fungal cell or fungal cell fragment may be derived from yeast that is grown continually or grown in a batch. Yeast grown continually is usually used for the production of ethanol for fuel purposes and is adapted to a high alcohol environment Such yeast is termed ethanol yeast or biofuel yeast of which Williams yeast is an example. Most preferably the fungal cell or fungal cell fragment is derived from biofuel yeast.

The composition may be applied to the epithelium exposed by a wound or of a mucous membrane.

The mucous membrane may be the membrane lining the oral cavity or buccal cavity, tongue, stomach, small intestine (duodenum or jejunum), large intestine (colon), rectum, vagina, cervix, nose, naso-pharynx, or pulmonary system (trachea, larynx, bronchi, and lungs). The mucous membrane may be the membrane lining of the digestive system of humans, domestic pets, and livestock.

The mucous membrane may be the lining of the oral cavity, buccal cavity or the tongue where the composition of the present invention may be for pharmaceutical use, oral health care, or as an over the counter (OTC) medicine. To deliver to the mouth or tongue, the composition of the present invention may be formulated as a powder, gel, spray, or tablet to treat for example, mouth ulcers, trench mount, gingivitis or canker sores. Compositions of the present invention may be formulated in the form of a dry or liquid (emulsion or suspension) syrup, a sachet, a chewable, a chewing gum, an orodispersible, a dispersible effervescent, a dispersible tablet, a compressed buccal tablet, a compressed sublingual tablet, a chewable tablet, and a lozenge. Chewable dosage forms for drug delivery are well known to the pharmaceutical industry.

The mucous membrane may be the membrane lining the pharynx/throat where the encapsulated product can be for pharmaceutical use or as an OTC medicine. The composition of the present invention may be formulated as a compressed sweet or boiled sweet To deliver the composition to

the naso-pharyngeal membranes, the composition of the present invention may be formulated as a powder, gel, spray or aerosol.

The mucous membrane may be the membrane lining the oesophagus or stomach, where the composition of the present invention can be for pharmaceutical use, nutriceutical applications, or as an OTC medicine. The composition of the present invention can be incorporated in a one- or two-part gelatin capsule or other similar material to aid swallowing and prevent premature release of the active in the mouth or on the surface of the tongue.

The mucous membrane may be the membrane lining the colon/rectum where the composition of the present invention may be for pharmaceutical use, or as an OTC medicine. Delivery of actives to the colon or rectum can also be achieved through the use composition of the present invention formulated as a suppository, ointment, cream, or gel.

The mucous membrane may be the membrane lining the nose, where the composition of the present invention may be for pharmaceutical use or as an OTC medicine. The composition of the present invention may be delivered as snuff, or as an aerosol delivered to the nose via nasal applicators as an aid for introducing the compositions of the present invention into the nasopharyngeal space of a patient.

The mucous membrane may be the membrane lining the pulmonary system (i.e larynx, trachea, bronchi, and lungs)

The mucous membrane may be the membrane lining the vagina/cervix where the composition of the present invention may be for pharmaceutical use or as an OTC medicine. The composition of the present invention may be formulated as a pessary, cream, ointment or gel. The mucous membrane may be the membrane lining the digestive system of humans, domestic pets, and livestock For example, delayed and controlled release of an active can take place when the active is released throughout the entire digestive system of humans. The biological membrane in this instance is the tunica mucosa, which lines the upper gastrointestinal tract, stomach, small intestine and colon.

Accordingly, the composition of the present invention may be formulated as a dry or liquid (emulsion or suspension) syrup, a sachet, a chewable, a chewing gum, an orodispersible, a dispersible effervescent, a dispersible tablet, a compressed buccal tablet, a compressed sublingual tablet, a chewable tablet, a melt-in-the-mouth, a lozenge, a paste, a powder, a gel, a tablet, a compressed sweet, a boiled sweet, a cream, a suppository, a snuff, a spray, an aerosol, a pessary, or an ointment.

In accordance with a further aspect of the present invention there is provided a method of manufacturing a composition as described hereinabove comprising contacting a capsule with the composition such that the composition is encapsulated by the capsule and retained passively

In accordance with a further aspect of the present invention, there is provided a wound dressing comprising a carrier and a composition as described hereinabove.

The carrier may comprise any material capable of being sanitized eg. By radiation, and non-irritating to damaged tissue. Preferably, the carrier comprises a non-woven synthetic material. More preferably, the carrier comprises polyester.

The carrier may be formed into strips or patches Strips or patches may be layered to promote retention of the composition between layers.

The composition may be disposed on said carrier or the carrier may be impregnated with said composition.

The wound dressing may be packaged to prevent contamination and/or damage whilst in storage or transit In one embodiment, the packaged wound dressing comprises a heat sealable envelope sealed around its perimeter with a wound dressing according to the present invention disposed therein.

The present invention further provides a method of producing an encapsulated material comprising treating a grown intact microbe such as a fungus or bacterium by contiguous contact with an encapsulatable material in liquid form The encapsulatable material being capable of diffusing into the microbial cell without causing total lysation thereof, and said treatment being carried out in the absence or presence of an organic lipid-extending substance (as defined in European Patent Specification No. 0085805) as solvent or microdispersant for the encapsulatable material and in the absence of a plasmolyser, whereby the material is absorbed by the microbe by diffusion across the microbial cell wall and is retained passively within the microbe (as described in European Patent Specification 0085805). The aforementioned prior methods rely either on special microbe cultivation conditions to enhance the microbial lipid content to a very high level or on the use of a lipid-extending substance, and the materials to be encapsulated must be either soluble in the microbial lipid or soluble or microdispersible in the lipid-extending substance, respectively.

In French Patent Specification No. 2179528 there is described a method of causing certain materials to be absorbed and/or fixed by microbes, in which a microbe such as pressed industrial yeast is treated with a plasmolyser, i.e a substance which causes contraction or shrinking of the microbial cytoplasm by exosmosis of cytoplasmic fluid, and then an aqueous solution of a material such as neodymium chloride, magnesium chloride or onion juice is added under certain conditions so that the aqueous material is absorbed in place of the extracted cytoplasmic fluid

In one embodiment, the fungal cell is in grown form, i e. it has been harvested from its culture medium, and is intact, i.e not lysed Suitably the microbe is alive, at least at the commencement of the treatment, however, a microbe which has been subjected to conditions (such as by irradiation of the microbe) to destroy its ability of propagate may be employed.

Preferably the capsule has a large size (cell size), for example of average diameter more than about 5 microns. Bacteria may have a smaller normal cell size of about 1 to 2 microns but may be cultivated to attain a larger size.

It is not necessary for the capsule to have a significant lipid content. Typically the lipid content may be not more than about 5%, for instance up to 3%, by dry weight of the microbe.

The encapsulatable material should be in liquid form during the treatment. It may be a liquid in its normal state, or it may be normally a solid in which case it should be dissolved or microdispersed in a solvent that is not miscible with the microbial lipid. Examples of suitable solvents are the lower alcohols such as methanol, ethanol and iso-propanol. The solvent may be removed after the encapsulation treatment, such as by spray-drying

In one embodiment, the composition further comprises a carrier for co-encapsulation with biocide or essential oil.

In one embodiment, where the composition comprises an essential oil and a biocidal compound, the carrier comprises the essential oil.

The encapsulatable material need not be soluble in any lipid forming part of the capsule

The method of encapsulation preferably comprises mixing the capsule with the composition in a liquid medium, especially an aqueous medium, to attain good dispersion and contact of the capsule with the composition. Accordingly, the composition may be mixed with an aqueous paste or slurry of the capsule, or the composition in a small quantity of water may be mixed with dry microbe. Preferably the composition forms an emulsion in the aqueous medium.

Encapsulation may be performed at normal ambient temperatures but preferably the temperature is elevated, at least during the initial stages, such as during at least the first 30 minutes, in order to expedite the encapsulation. A suitable elevated temperature may be in the range 35 to 70°C, more preferably 45-60°C.

The encapsulation may be observed microscopically as one or more globules of the composition inside the capsule. This may take a few hours.

In one embodiment, the capsule may be pretreated at an elevated temperature and/or with a proteolytic enzyme and/or with a chemical such as sodium hydroxide or a magnesium salt to enhance permeability prior to or in some cases during the encapsulation process. Such pretreatment may be carried out by incubating the microbe in water at an elevated temperature The microbe may then be mixed with the material to be encapsulated at a lower temperature.

After encapsulation, the capsule may be treated to soften it in order to facilitate subsequent release of the encapsulated material, such as by treatment with a proteolytic enzyme or an alkali, or it may be treated to harden it in order to prevent premature liberation of the encapsulated material, such as by treatment with a dilute aqueous aldehyde solution The encapsulated material may be released from the capsules when desired by, for instance, chemical, biodegradation or mechanical rupture of the microbial cell wall, and/or by subjecting the capsules to an environment in which the material diffuses gradually out through pores in the capsule and/or contacting the fungal cell wall or fragment thereof with epithelial cells and/or contacting the capsules with a compound that breaks down or disrupts the structure of cell membrane.

Capsules produced by the invention give rise to controlled release characteristics; for example when the release of the encapsulated material is delayed or prolonged by a slow or gradual rupture of the capsule or slow diffusion therefrom providing a sustained treatment.

Specific embodiments of the present invention will now be described, by way of example only, with reference to the following examples

1.0 Selected staphylococcal strains

Strains of staphylococcus (n=36) are as follows: *S aureus* (n=2), *S. epidermidis* (n=2), *S. saprophyticus* (n=1), *S. haemolyticus* (n=1), Methicillin sensitive *S. aureus* (MSSA) (n=6), Methicillin resistant *S. aureus* (MRSA) (n=9), Epidemic methicillin resistant *S. aureus* (EMRSA) (n=15). Within the EMRSA strains selected, 3 strains are currently causing major problems within hospitals internationally

1.1 . Selected essential oils

Manuka oil (*leptospermum Scoparium*), Geranium (Egypt) (*Pelargonium graveolens*), Lavender Eastern Europe (*Lavandula angustifolia*), Lemongrass (East Indian) (*Cymbopogon flexuosus*), Tea tree (*Melaleuca alternifolia*).

1 1.1 Preparation of encapsulated essential oils

Individual essential oils were encapsulated in washed Williams yeast. This was achieved by combining essential oil, washed Williams yeast and tap water in ratios of 1.2:4. The three components were mixed for 4 hours at 40°C and then spray dried at 170 – 180°C. This resulted in a fine powder of encapsulated essential oils. The volume of essential oil retained within the yeast was determined by gas chromatography (µl/mg).

1 1.2 Preparation of essential oil and encapsulated essential oil combinations

Combinations of essential oil and encapsulated essential oil mixtures were performed in ratios of 50.50, 75:25 and 25:75. To obtain an equivalent of the essential oil combinations in the encapsulated product, the combinations were carried out based upon the weight of the product.

The combinations were as follows: Manuka + Geranium/ Geranium + Lavender/ Manuka + Lavender/ Geranium + Lemongrass/ Manuka + Lemongrass/ Geranium + Tea tree/ Manuka + Tea tree/ Lavender + Lemongrass/ Lemongrass + Tea tree/ Lavender + Tea tree

- Example of essential oil combinations: 75. 25 Lemongrass and Lavender
  - 1600 µl of essential oil (1200µl Lemongrass: 400 µl Lavender) + 400 µl of AAB = 2 ml of 80 % combined oil
  - 1 ml of 80 % used to create dilutions and 1 ml added to 19 ml of STA = 4 % oil

- Example of encapsulated essential oil combinations: 75· 25 Manuka and Tea tree
  - 2286 mg of Manuka (750 µl oil) + 957 mg of Tea tree (250 µl oil) added to 9 ml of AAB = 10 ml of 10 % oil

- 1 ml of 10 % used to created dilutions and 1 ml added to 19 ml of STA = 0 5 % oil

2 Estimation of the Minimum inhibitory concentrations (MIC) of all strains against single and combined essential oils (Direct contact)

Each individual essential oil and essential oil combination (see section 1 1 2) was diluted from 80% to 0 63% using antibiotic assay broth (AAB) Each dilution (1 ml) was then vortex mixed with 19 ml of molten sensitivity test agar (STA) and dispensed into individual petri dishes Plates were allowed to set then dried for 30 minutes After addition of the dilutions to the STA, each essential oil or essential oil combination resulted in a dilution of 4% to 0.031%

An overnight broth culture (ONBC) of each bacterial strain was diluted 1/10 using AAB Each strain was then placed onto the surface of the STA containing the essential oil and essential oil combinations, using a multi-point inoculator Plates were dried for 20 minutes and then incubated for 24 hours at 37°C The MIC of each strain was determined as the first plate within the dilution series showing no growth of the organism

RESULTS

The MIC of single essential oils against all strains of staphylococci.

Strain	Essential oils				
	Lavender	Geranium	Lemongrass	Manuka	Tea tree
Oxford S aureus NCTC 6571	1%	2%	0.5%	0 125%	0 125%
S aureus NCBC 11882	1%	1%	1 0%	0 125%	0 250%
S. epidermidis NCTC 11047	1%	1%	0 5%	0.125%	0 250%
S epidermidis NCTC 7944	1%	1%	1 0%	0.5%	0 250%
S saprophyticus NCIMB 8711	1%	1%	1.0%	0 125%	0 125%
S haemolyticus NCTC 11042	1%	1%	1.0%	0 125%	0 125%
Strain T1 MSSA	1%	1%	1.0%	0.125%	0 125%
Strain T4 MSSA	1%	1%	1 0%	0 125%	0.062%
MSSA (4)	1%	2%	1 0%	0 125%	0 125%
MSSA (46)	1%	1%	1.0%	0.125%	0 250%
MSSA (47)	1%	1%	1.0%	0.125%	0 125%
MSSA (48)	1%	2%	1.0%	0.125%	0.125%
MRSA 11	1%	2%	1 0%	0.5%	0 062%
MRSA 12	1%	1%	1.0%	0.125%	0 062%
MRSA 13	1%	1%	1.0%	0.25%	0 125%
MRSA 14	1%	1%	1 0%	0 125%	0 125%
MRSA 15	1%	2%	1.0%	0 25%	0 125%

MRSA 16	1%	2%	0 5%	0 125%	0 125%
MRSA 17	1%	2%	1 0%	0 125%	0 125%
MRSA 20	1%	2%	1 0%	0 125%	0 125%
MRSA 26	1%	1%	1 0%	0 125%	0 250%
EMRSA m97 271 031 phage group 1	1%	2%	1 0%	0 125%	0 250%
EMRSA m97 271 038 phage group 2	1%	1%	1 0%	0 125%	0 125%
EMRSA j95 922 phage group 3	1%	1%	1 0%	0 125%	0.125%
EMRSA m97271052 phage group 4	1%	2%	1.0%	0.125%	0 125%
EMRSA m972 71041 phage group 5	1%	2%	0.5%	0 125%	0 062%
EMRSA m97 271 088 phage group 6	1%	2%	1 0%	0 125%	0 250%
EMRSA m97 271 047 phage group 8	1%	2%	1 0%	0.125%	0 125%
EMRSA m972 710 40 phage group 9	1%	1%	1 0%	0.125%	0 062%
EMRSA m97 271 032 phage group 10	1%	1%	1.0%	0 125%	0 125%
EMRSA m97 271 036 phage group 11	1%	1%	1 0%	0.125%	0.062%
EMRSA m97 271 042 phage group 12	1%	1%	1 0%	0 125%	0 250%
EMRSA m97 271 064 phage group 14	1%	1%	1.0%	0 125%	0 062%
EMRSA g96 139 515 phage group 15	1%	1%	1.0%	0 5%	0.062%
EMRSA g96 138 744 phage group 16	1%	2%	0.5%	0 125%	0.125%
EMRSA g96 136 210 phage group 17	1%	1%	1 0%	0 125%	0.062%

The standard deviation for each was zero

09 12 04

	Essential oil combinations									
Strains	1	2	3	4	5	6	7	8	9	10
Oxford S aureus NCTC 6571	0 062%	0 5%	0 125%	0 062%	0 5%	0.125%	0.062%	0 5%	0 5%	0 25%
S aureus NCBC 11882	0 01%	0 5%	0 125%	0 062%	1%	0 125%	0 062%	0 5%	0 5%	0 5%
S. epidermidis NCTC 11047	0 062%	0 5%	0 125%	0 062%	1%	0 125%	0 062%	0 5%	0 5%	0 5%
S epidermidis NCTC 7944	0 062%	0.5%	0 125%	0 062%	1%	0 125%	0.5%	0 5%	0 125%	0 5%
S saprophyticus NCIMB 8711	0 25%	0 5%	0 125%	0 062%	1%	0 125%	0 062%	0 5%	0 5%	0 5%
S haemolyticus NCTC 11042	0 01%	0 5%	0 125%	0.062%	1%	0 031%	0 5%	0 031%	0 5%	0 5%
Strain T1 MSSA	0 01%	0.5%	0 125%	0 062%	1%	0 062%	0.5%	0 062%	0 5%	0 5%
Strain T4 MSSA	0 01%	0 25%	0 125%	0 062%	1%	0 125%	0 062%	0 031%	0 25%	0 5%
MSSA (4)	0 031%	0 5%	0 125%	0 062%	0 5%	0 031%	0 062%	0 031%	0 25%	0 5%
MSSA (46)	0.031%	0 5%	0.125%	0 01%	1%	0 125%	0 062%	0 062%	0 5%	0 5%
MSSA (47)	0 031%	0 5%	0 125%	0.062%	0 5%	0 125%	0.062%	0 5%	0 062%	0 5%
MSSA (48)	0 031%	0 5%	0 125%	0.01%	0 5%	0.125%	0 062%	0 5%	0 5%	0 5%
MRSA 11	0 031%	0 5%	0.125%	0 01%	1%	0 125%	0 25%	0 5%	0 5%	0 5%
MRSA 12	0 031%	0.5%	0 125%	0 062%	0 5%	0 062%	0 031%	0 062%	0 062%	0 5%
MRSA 13	0 125%	0 5%	0 125%	0.01%	1%	0 125%	0 5%	0 5%	0 5%	0 5%
MRSA 14	0 031%	0 5%	0 125%	0 01%	1%	0 061%	0 062%	0 031%	0 25%	0 5%
MRSA 15	0 031%	0 5%	0 125%	0 062%	1%	0 061%	0.062%	0 031%	0 25%	0 25%
MRSA 16	0 031%	0 5%	0 125%	0 062%	0 5%	0 061%	0 062%	0.062%	0 061%	0 25%
MRSA 17	0 031%	0 5%	0 125%	0 01%	1%	0 061%	0 5%	0.062%	0 5%	0 5%
MRSA 20	0 031%	0 5%	0 125%	0 01%	1%	0 125%	0.062%	0 031%	0.5%	0.5%
MRSA 26	0 031%	0 062%	0 125%	0 062%	0 5%	0 125%	0 062%	0 5%	0 5%	0 5%
EMRSA m97 271 031 P grp 1	0 125%	0 5%	0 125%	0.062%	0 25%	0 125%	0 031%	0 5%	0 062%	0 5%
EMRSA m97 271 038 P grp 2	0 125%	0 5%	0 125%	0 062%	0 25%	0 125%	0 031%	0 5%	0 062%	0 25%
EMRSA j95 922 P grp 3	0 125%	0 5%	0 125%	0 062%	0.25%	0 125%	0.031%	0 5%	0.031%	0 25%
EMRSA m97271052 P. grp 4	0 062%	0 5%	0 125%	0 062%	0 5%	0 125%	0 5%	0 062%	0 031%	0 5%
EMRSA m972 71041 P grp 5	0 01%	0 062%	0 125%	0.062%	0 25%	0 125%	0 5%	0 5%	0 031%	0.5%
EMRSA m97 271 088 P grp 6	0 062%	0.5%	0 125%	0 062%	0 5%	0 125%	0 062%	0 5%	0 031%	0 5%
EMRSA m97 271 047 P grp 8	0 062%	0 5%	0 125%	0 062%	0 5%	0 125%	0 062%	0 5%	0 031%	0 5%
EMRSA m972 710 40 P grp 9	0 062%	0 5%	0 125%	0 125%	1 00%	0 031%	0 062%	0 5%	0 25%	0 5%
EMRSA m97 271 032 P grp 10	0 01%	0 5%	0 125%	0 125%	0 5%	0 031%	0 062%	0 5%	0 25%	0 5%
EMRSA m97 271 036 P grp 11	0 062%	0 5%	0 125%	0 125%	0 5%	0 125%	0 062%	0 062%	0 25%	0 5%
EMRSA m97 271 042 P grp 12	0 062%	0 5%	0 125%	0 125%	0 5%	0 125%	0 062%	0 062%	0 031%	0 5%
EMRSA m97 271 064 P grp 14	0 062%	0 5%	0 125%	0 125%	0 5%	0 125%	0 031%	0 062%	0 031%	0 3%
EMRSA g96 139 515 P grp 15	0 062%	0.5%	0 125%	0 125%	0 5%	0 125%	0 5%	0 062%	0 031%	0 5%
EMRSA g96 138 744 P grp 16	0 031%	0 5%	0 125%	0 125%	0.5%	0 125%	0 062%	0 031%	5 0%	0 5%
EMRSA g96 136 210 P grp 17	0 031%	0 5%	0 125%	0 125%	0 5%	0 031%	0 062%	0 031%	0 062%	0 5%

The MIC of essential oil combinations against all strains of staphylococci

KEY Essential oils	No
50 % Lemongrass 50 % Geranium	1
50 % Manuka, 50 % Geranium	2
50 % Tea tree, 50 % Lemongrass	3
50 % Tea tree, 50 % Lavender	4
50 % Tea tree, 50 % Geranium	5
50 % Manuka, 50 % Tea tree	6
50 % Manuka, 50 % Lemongrass	7
50 % Manuka, 50 % Lavender	8
50 % Lemongrass 50 % Lavender	9
50 % Geranium 50 % Lavender	10

The standard deviation for each was zero

Strains	essential oils combinations									
	1	2	3	4	5	6	7	8	9	10
Oxford S aureus NCTC 6571	0.062%	0 031%	1%	1%	2%	0 031%	0 031%	0 062%	0 25%	0 125%
S aureus NCBC 11882	0.062%	0 031%	1%	1%	2%	0 5%	0 031%	0 031%	0 5%	0 125%
S epidermidis NCTC 11047	0 062%	0 031%	1%	1%	2%	0 031%	0.031%	0 031%	0 5%	0 125%
S epidermidis NCTC 7944	0 062%	0 031%	1%	0 125%	2%	0 031%	0 031%	0 031%	0.5%	0 125%
S saprophyticus NCIMB 8711	0 5%	0 031%	1%	0 125%	2%	0 031%	0 031%	0 031%	0 250%	0 125%
S haemolyticus NCTC 11042	0 5%	0 031%	1%	1%	2%	0 031%	0 031%	0 062%	0 5%	0 125%
Strain T1 MSSA	0 031%	0 031%	1%	1%	2%	0 01%	0 031%	0 125%	0 5%	0 125%
Strain T4 MSSA	0 031%	0 031%	1%	1%	2%	0 031%	0 031%	0 125%	0 5%	0 125%
MSSA (4)	0 5%	0 031%	1%	1%	2%	0 031%	0 031%	0 062%	0 5%	0 031%
MSSA (46)	0 031%	0 031%	1%	1%	2%	0.031%	0 031%	0 062%	0.5%	0 125%
MSSA (47)	0 01%	0 031%	1%	0 125%	2%	0.031%	0.031%	0 125%	1%	0 125%
MSSA (48)	0 5%	0 031%	1%	1%	2%	0 031%	0 031%	0 062%	0 5%	0 125%
MRSA 11	0 5%	0 031%	1%	1%	2%	0 031%	0 031%	0 125%	2 0%	0 25%
MRSA 12	0 5%	0.031%	1%	1%	2%	0 01%	0 031%	0 125%	0 5%	0 062%
MRSA 13	0 5%	0 031%	1%	1%	2%	0 01%	0.031%	0 031%	2%	0 25%
MRSA 14	0 5%	0 01%	1%	1%	2%	0 01%	0 031%	0 031%	0 5%	0 125%
MRSA 15	0 5%	0 01%	0.5%	1%	2%	0.01%	0 031%	0 031%	0 25%	0.062%
MRSA 16	0 062%	0 031%	1%	0 125%	2%	0.031%	0 031%	0 031%	0 25%	0 062%
MRSA 17	0 062%	0 031%	1%	0 125%	2%	0.031%	0.031%	0 125%	1%	0 125%
MRSA 20	0 062%	0 031%	1%	0 125%	2%	0 031%	0 031%	0 125%	0 5%	0.062%
MRSA 26	0 062%	0 031%	1%	0 125%	2%	0 031%	0 031%	0.125%	1%	0 062%
EMRSA m97 271 031 P. grp 1	0 01%	0.031%	1%	0 125%	2%	0 031%	0.031%	0 125%	1%	0.125%
EMRSA m97 271 038 P grp 2	0 01%	0.031%	1%	0.5%	2%	0 031%	0 031%	0 125%	0 125%	0 062%
EMRSA j95 922 P grp 3	0.01%	0.01%	1%	0 5%	2%	0 031%	0.031%	0 125%	0 25%	0 062%
EMRSA m97271052 P grp 4	0 01%	0 031%	1%	0.125%	2%	0 031%	0 031%	0 125%	1%	0 062%
EMRSA m972 71041 P grp 5	0 01%	0 031%	1%	1%	2%	0 01%	0 031%	0.125%	1%	0 062%
EMRSA m97 271 088 P.grp 6	0 01%	0 031%	1%	1%	2%	0 031%	0 031%	0 125%	1%	0.062%
EMRSA m97 271 047 P grp 8	0 01%	0 031%	1%	1%	2%	0 031%	0.031%	0 125%	0.25%	0 062%
EMRSA m972 710 40 P grp 9	0 01%	0 031%	1%	0 5%	2%	0.031%	0 031%	0 125%	0 5%	0 062%
EMRSA m97 271 032 P grp 10	0 01%	0.031%	1%	1%	2%	0 031%	0.031%	0 125%	0 5%	0 125%
EMRSA m97 271 036 P grp 11	0 01%	0 031%	1%	0.125%	2%	0 031%	0 031%	0 125%	0 5%	0 125%
EMRSA m97 271 042 P.grp 12	0 01%	0 031%	1%	0.125%	2%	0 031%	0 031%	0 125%	0.5%	0 125%
EMRSA m97 271 064 P grp 14	0 01%	0 031%	1%	0.125%	2%	0 031%	0.031%	0 125%	0 25%	0 062%
EMRSA g96 139 515 P grp 15	0 01%	0 031%	1%	0 5%	2%	0 031%	0 031%	0 125%	0 5%	0 125%
EMRSA g96 138 744 P grp 16	0 01%	0 031%	1%	0.5%	2%	0 031%	0 031%	0 125%	0 5%	0 125%
EMRSA g96 136 210 P grp 17	0 01%	0 031%	1%	1%	2%	0 031%	0 031%	0 125%	0 5%	0.062%

The MIC of essential oil combinations against all strains of staphylococci

KEY Essential oils	No
75 % Lemongrass 25 % Geranium	1
75 % Manuka, 25 % Geranium	2
75 % Tea tree, 25 % Lemongrass	3
75 % Tea tree, 25 % Lavender	4
75 % Tea tree, 25 % Geranium	5
75 % Manuka, 25 % Tea tree	6
75 % Manuka, 25 % Lemongrass	7
75 % Manuka, 25 % Lavender	8
75 % Lemongrass 25 % Lavender	9
75 % Geranium 25 % Lavender	10

The standard deviation for each was zero

Strains	Essential oil combinations									
	1	2	3	4	5	6	7	8	9	10
Oxford S aureus NCTC 6571	0 062%	0 062 %	0 5%	0 25%	0 5%	1%	0 062%	0 5%	0 25%	0 5%
S aureus NCBC 11882	0 5%	0 25%	1%	1%	0 5%	1%	0 25%	1%	0 5%	1%
S epidermidis NCTC 11047	0 13%	0 25%	0 25%	1%	0 5%	1%	0 25%	1%	0 5%	1%
S epidermidis NCTC 7944	0 25%	0 5%	0 25%	1%	0 5%	1%	0 5%	1%	0 5%	1%
S saprophyticus NCIMB 8711	0 5%	0 5%	0.5%	1%	0 5%	1%	0 5%	1%	0 5%	1%
S haemolyticus NCTC 11042	0 5%	0 5%	1%	1%	0 5%	1%	0 5%	1%	0 5%	1%
Strain T1 MSSA	0 5%	0.5%	1%	1%	0 5%	2%	0.5%	1%	0 5%	1%
Strain T4 MSSA	0.5%	0 25%	0 5%	1%	0 5%	1%	0 25%	1%	0 5%	1%
MSSA (4)	0.5%	0 25%	0 5%	1%	0 5%	1%	0 25%	1%	0 5%	0 5%
MSSA (46)	0 5%	0 5%	1%	1%	0 5%	1%	0 5%	1%	0 5%	0 5%
MSSA (47)	0.5%	0.25%	0 5%	1%	0 5%	2%	0 25%	1%	0 5%	0 5%
MSSA (48)	0 5%	0 5%	0.5%	1%	0.5%	1%	0.5%	1%	0 5%	0 5%
MRSA 11	0 5%	0 5%	1%	1%	0.5%	2%	0 5%	1%	0.5%	0 5%
MRSA 12	0 5%	0 5%	1%	1%	0 5%	1%	0 5%	1%	0 5%	0.5%
MRSA 13	0 5%	0.5%	1%	1%	0.5%	2%	0 5%	1%	0.5%	0 5%
MRSA 14	0 5%	0 25%	0 5%	1%	0.5%	1%	0.25%	1%	0 5%	0 5%
MRSA 15	0 5%	0 5%	0 5%	1%	0 5%	2%	0 5%	1%	0 5%	0.5%
MRSA 16	0 25%	0.25%	0 5%	1%	0 5%	1%	0.25%	1%	0 25%	0 25%
MRSA 17	0 5%	0 5%	1%	1%	0 5%	2%	0 5%	1%	0.5%	1%
MRSA 20	0 5%	0 5%	0 5%	1%	0.5%	2%	0.5%	1%	0 5%	1%
MRSA 26	0 5%	0 5%	0 5%	1%	0 5%	2%	0 5%	1%	0.5%	0 5%
EMRSA m97 271 031 P grp 1	0 25%	0 25%	0 5%	0 5%	0 5%	1%	0 25%	1%	0 5%	0 5%
EMRSA m97 271 038 P grp 2	0 25%	0 25%	0.5%	1%	0.5%	1%	0 25%	1%	0 5%	0.5%
EMRSA j95 922 P grp 3	0 25%	0 25%	0.5%	1%	0 5%	2%	0 25%	1%	0 5%	0 5%
EMRSA m97271052 P. grp 4	0 25%	0.25%	0 5%	1%	0 5%	2%	0 25%	1%	0 5%	0 5%
EMRSA m972 71041 P grp 5	0.5%	0.5%	0 5%	1%	0.5%	2%	0 5%	1%	0 5%	0 5%
EMRSA m97 271 088 P grp 6	0 5%	0 25%	0 5%	1%	0.5%	2%	0 5%	1%	0 5%	0.5%
EMRSA m97 271 047 P grp 8	0 5%	0 5%	0 5%	1%	0 5%	2%	0.5%	1%	0 5%	0 5%
EMRSA m972 710 40 P grp 9	0 5%	0 5%	0 5%	1%	0 5%	2%	0 5%	1%	1%	1%
EMRSA m97 271 032 P.grp 10	0 5%	0 5%	0 5%	1%	0 5%	2%	0 5%	1%	0 5%	0 5%
EMRSA m97 271 036 P grp 11	0 25%	0 25%	0 5%	1%	0 5%	2%	0.25%	1%	0 5%	0 5%
EMRSA m97 271 042 P grp 12	0 5%	0.5%	0 5%	1%	0 5%	2%	0 5%	1%	0 5%	0 5%
EMRSA m97 271 064 P grp 14	0 5%	0.25%	0 5%	1%	0 5%	2%	0 25%	1%	0 5%	0 5%
EMRSA g96 139 515 P grp 15	0 5%	0 5%	0 5%	1%	0 5%	2%	0 5%	1%	0 5%	0 5%
EMRSA g96 138 744 P grp 16	0 5%	0 5%	0 5%	1%	0 5%	2%	0 5%	1%	0 5%	0 5%
EMRSA g96 136 210 P grp 17	0 5%	0 5%	0 5%	1%	0 5%	2%	0 5%	1%	0 5%	0 5%

The MIC of essential oil combinations against all strains of staphylococci

Key Essential oils	No
25 % Lemongrass 75 % Geranium	1
25 % Manuka, 75 % Geranium	2
25 % Tea tree, 75 % Lemongrass	3
25 % Tea tree, 75 % Lavender	4
25 % Tea tree, 75 % Geranium	5
25 % Manuka, 75 % Tea tree	6
25 % Manuka, 75 % Lemongrass	7
25 % Manuka, 75 % Lavender	8
25 % Lemongrass 75 % Lavender	9
25 % Geranium 75 % Lavender	10

The standard deviation for each was zero

IN BRIEF

The MIC of essential oils and essential oil combinations that gave full inhibition of all strains of staphylococci.

Essential oil	MIC (%)
Lavender	1
Geranium	2
Lemongrass	1
Manuka	0.50
Tea tree	0 25

Essential oil combinations	50:50 (MIC %)	75:25 (MIC %)	25:75 (MIC %)
Manuka and Geranium	0.5	0.031	0.5
Manuka and Tea tree	0.125	0 031 (0.5*)	2
Manuka and Lemongrass	0.5	0.031	0.5
Manuka and Lavender	0.5	0 25	1
Tea tree and Lemongrass	0.125	1	1
Tea tree and Lavender	0.125	1	1
Tea tree and Geranium	1	2	0 5
Lemongrass and Lavender	0 5	0.125	0 5 (1*)
Lemongrass and Geranium	0.125 (0.25*)	0 5	0 5
Geranium and Lavender	0.5	2	1

* This MIC is for one strain only

CONCLUSION

Essential oils and essential oil combinations that gave the best results were:

75 % Manuka and 25 % Geranium

75 % Manuka and 25 % Tea tree

75% Manuka and 25 % Lemongrass

- Combinations of some essential oils were more effective than when the individual essential oils were used singly.

3 Estimation of the Minimum inhibitory concentration (MIC) of staphylococci against combined encapsulated essential oils (direct contact).

The encapsulated essential oil combinations are as described hereinabove. Each encapsulated essential oil combination was diluted from 80% to 0.63% using antibiotic assay broth (AAB). Each dilution (1 ml) was then vortex mixed with 19 ml of molten sensitivity test agar (STA) and dispensed into individual petri dishes. Plates were allowed to set than dried for 30 minutes. After addition of the dilutions to the STA, each encapsulated essential oil combination resulted in a dilution of 4% to 0 031%.

An overnight broth culture (ONBC) of each bacterial strain was diluted 1/10 using AAB. Each strain was then placed onto the surface of the STA containing the encapsulated essential oil combinations, using a multi-point inoculator Plates were dried for 20 minutes then incubated for 24 hours at 37°C The MIC of each strain was determined as the first plate within the dilution series showing no growth of the organism

RESULTS

<u>The MIC of encapsulated essential oil combinations against all staphylococcal strains</u>

Strains	Essential oil combinations								
	Manuka and geranium 75 25			Manuka and tea tree 75 25			Manuka and lemongrass 75 25		
	1	2	Average	1	2	Average	1	2	Average
Oxford S aureus NCTC 6571	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 063%	0 125%	0 125%
S aureus NCBC 11882	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 063%	0 125%	0 125%
S epidermidis NCTC 11047	0 063%	0 063%	0 063%	0 25%	0 25%	0 25%	0 125%	0 125%	0 125%
S epidermidis NCTC 7944	0 063%	0 063%	0 063%	0 25%	0 25%	0 25%	0 125%	0 125%	0 125%
S saprophyticus NCIMB 8711	0 063%	0 063%	0 063%	0 25%	0 25%	0 25%	0 063%	0 125%	0 125%
S haemolyticus NCTC 11042	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 063%	0 125%	0 125%
Strain T1 MSSA	0 063%	0 063%	0 063%	0 25%	0 25%	0 25%	0 125%	0 125%	0 125%
Strain T4 MSSA	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 125%	0 125%	0 125%
MSSA (4)	0 063%	0 125%	0 125%	0 25%	0 125%	0 25%	0.063%	0 063%	0 063%
MSSA (46)	0 063%	0 063%	0 063%	0 125%	0 125%	0 125%	0 125%	0 125%	0 125%
MSSA (47)	0.063%	0 125%	0 125%	0 125%	0.125%	0 125%	0 063%	0.063%	0.063%
MSSA (48)	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 125%	0 125%	0 125%
MRSA 11	0 063%	0 063%	0 063%	0 25%	0 125%	0 25%	0 125%	0 125%	0 125%
MRSA 12	0 063%	0 063%	0 063%	0 125%	0 125%	0.125%	0 125%	0 125%	0 125%
MRSA 13	0 063%	0 063%	0.063%	0 125%	0 125%	0 125%	0 125%	0 125%	0.125%
MRSA 14	0 063%	0 063%	0 063%	0 125%	0 125%	0 125%	0 125%	0 125%	0 125%
MRSA 15	0 063%	0 063%	0 063%	0 25%	0 25%	0 25%	0 125%	0 125%	0 125%
MRSA 16	0.063%	0 125%	0 125%	0 125%	0 125%	0 125%	0 010%	0 032%	0 032%
MRSA 17	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 125%	0 125%	0 125%
MRSA 20	0 063%	0 063%	0 063%	0 125%	0 125%	0 125%	0 063%	0 063%	0.063%
MRSA 26	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 125%	0 125%	0 125%
EMRSA m97 271 031 P grp 1	0 063%	0 125%	0 125%	0.125%	0 125%	0 125%	0.063%	0 125%	0 125%
EMRSA m97 271 038 P grp 2	0 063%	0 063%	0 063%	0 25%	0 125%	0 125%	0 063%	0 125%	0 125%
EMRSA j95 922 P grp 3	0 063%	0 063%	0 063%	0 25%	0 25%	0 25%	0 125%	0.125%	0 125%
EMRSA m97271052 P grp 4	0 063%	0 063%	0 063%	0.125%	0 125%	0 125%	0 125%	0 125%	0.125%
EMRSA m972 71041 P grp 5	0 063%	0 063%	0 063%	0 125%	0 125%	0 125%	0 125%	0.125%	0 125%
EMRSA m97 271 088 P grp 6	0 063%	0 063%	0 063%	0 125%	0 125%	0 125%	0 063%	0 063%	0 063%
EMRSA m97 271 047 P grp 8	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 063%	0 125%	0 125%
EMRSA m972 710 40 P grp 9	0.063%	0 125%	0 125%	0 125%	0 25%	0 25%	0 063%	0.125%	0 125%
EMRSA m97 271 032 P grp 10	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 125%	0 125%	0 125%
EMRSA m97 271 036 P grp 11	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 125%	0 125%	0 125%
EMRSA m97 271 042 P grp 12	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 125%	0 125%	0 125%
EMRSA m97 271 064 P grp 14	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 125%	0 125%	0 125%
EMRSA g96 139 515 P grp 15	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 125%	0 125%	0 125%
EMRSA g96 138 744 P grp 16	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 125%	0.125%	0 125%
EMRSA g96 136 210 P grp 17	0 063%	0 063%	0 063%	0 125%	0 25%	0 25%	0 125%	0 125%	0.125%

The highest MIC of differing MIC's was used as an average. A difference in MIC's may have been due to experimental error.

CONCLUSION

- All encapsulated combinations were effective at inhibiting growth of all strains of staphylococci.
- Combinations of some encapsulated essential oils were more effective than when using the individual encapsulated essential oils alone.
- Combined encapsulated essential oils had a higher MIC than the combined non-encapsulated essential oils However, the encapsulated products may be retaining the oils within the yeast cells and potentially prolonging release

4. Assessment of the vapours of single and combined essential oils against strains of staphylococci (Vapour phase)

Neat (100%) individual essential oils and essential oil combinations (see section 1.1.2) (20µl) were placed onto individual 6 mm filter paper disks and the discs placed onto the lids of individual petri dishes

An overnight broth culture (ONBC) of each bacterial strain was diluted 1/100 using AAB and swabbed over the surface of a STA plate Plates were dried for 20 minutes. The disks were then placed into the petri dish lid and the lid placed onto the petri dish. All plates were incubated for 24 hours at 37°C. The ZOI of each strain was determined by measuring the area of bacterial clearing (diameter, mm).

- 10 strains were initially used to screen if the essential oils or combinations were effective If shown to be effective the oil was assessed against all strains.

RESULTS

(1) The ZOI of 10 strains of staphylococcus tested against the vapours of single essential oils

Strain	Essential oils		
	Lavender	Geranium	Manuka
	*	*	*
Oxford S. aureus NCTC 6571	FG	FG	FG
S. epidermidis NCTC 11047	FG	FG	FG
S. haemolyticus NCTC 11042	FG	FG	FG
Strain T4 MSSA	FG	FG	FG
MRSA 12	FG	FG	FG
MRSA 13	FG	FG	FG
MRSA 14	FG	FG	FG
EMRSA m97 271 064 P.grp 14	FG	FG	FG
EMRSA g96 139 515 P. grp 15	FG	FG	FG
EMRSA g96 138 744 P. grp 16	FG	FG	FG

FG= Full growth (no area of clearing)

* The standard deviation of all was zero.

All three oils were not effective so their effect on all strains was not assessed.

(2) The ZOI of 10 strains of staphylococcus against the vapours of single essential oils

Strains	Essential oils							
	Lemongrass				Tea tree			
	1	2	Average	SD±	1	2	Average	SD±
Oxford S aureus NCTC 6571	20	21	20.5	0 71	15	17	16	1 41
S. aureus NCBC 11882	25	24	24 5	0.71	15	16	15 5	0.71
S. epidermidis NCTC 11047	85	87	86	1 41	FG	FG	FG	FG
S. epidermidis NCTC 7944	46	48	47	1.41	FG	FG	FG	FG
S. saprophyticus NCIMB 8711	42	45	43.5	2.12	FG	FG	FG	FG
S. haemolyticus NCTC 11042	20	16	18	2.83	10	10	10	0.00
Strain T1 MSSA	30	31	30.5	0.71	FG	FG	FG	FG
Strain T4 MSSA	40	43	41.5	2.12	10	12	11	1 41
MSSA (4)	55	54	54.5	0 71	16	15	15.5	0 71

MSSA (46)	27	25	26	1 41	FG	FG	FG	FG
MSSA (47)	47	43	45	2 83	15	15	15	0 00
MSSA (48)	34	39	36 5	3 54	10	12	11	1 41
MRSA 11	32	33	32 5	0 71	11	16	13 5	3 54
MRSA 12	30	33	31 5	2 12	7	10	8 5	2 12
MRSA 13	33	29	31	2 83	FG	FG	FG	FG
MRSA 14	35	32	33 5	2 12	9	10	9 5	0 71
MRSA 15	34	36	35	1 41	16	18	17	1 41
MRSA 16	36	39	37.5	2 12	23	25	24	1 41
MRSA 17	21	20	20 5	0 71	25	29	27	2 83
MRSA 20	21	19	20	1 41	16	14	15	1 41
MRSA 26	30	26	28	2 83	FG	FG	FG	FG
EMRSA m97 271 031 phage group 1	47	49	48	1 41	27	22	24 5	3 54
EMRSA m97 271 038 phage group 2	30	32	31	1 41	FG	FG	FG	FG
EMRSA j95 922 phage group 3	55	52	53 5	2 12	17	18	17 5	0 71
EMRSA m97271052 phage group 4	65	61	63	2 83	25	26	25 5	0 71
EMRSA m972 71041 phage group 5	50	49	49 5	0 71	25	26	25 5	0 71
EMRSA m97 271 088 phage group 6	45	49	47	2 83	FG	FG	FG	FG
EMRSA m97 271 047 phage group 8	27	23	25	2 83	12	10	11	1 41
EMRSA m972 710 40 phage group 9	50	42	46	5 66	10	11	10 5	0 71
EMRSA m97 271 032 phage group 10	29	26	27.5	2 12	15	17	16	1 41
EMRSA m97 271 036 phage group 11	47	46	46 5	0 71	11	9	10	1 41
EMRSA m97 271 042 phage group 12	43	48	45 5	3 54	21	25	23	2 83
EMRSA m97 271 064 phage group 14	25	27	26	1 41	8	10	9	1 41
EMRSA g96 139 515 phage group 15	30	34	32	2.83	14	12	13	1 41
EMRSA g96 138 744 phage group 16	60	62	61	1 41	FG	FG	FG	FG
EMRSA g96 136 210 phage group 17	38	36	37	1 41	18	12	15	4 24

FG = Full growth (no area of clearing)

(1) The ZOI of 10 strains of staphylococcus against combined essential oils

	Essential oil combinations							
Essential oil	1	2			3	4	5	6
Strain	*	1 2	Average	SD±	*	*	*	*
Oxford S aureus NCTC 6571	FG	12 15	13.5	2 121	FG	FG	FG	FG
S epidermidis NCTC 11047	FG	FG FG	FG	FG	FG	FG	FG	FG
S haemolyticus NCTC 11042	FG	FG FG	FG	FG	FG	FG	FG	FG
Strain T4 MSSA	FG	FG FG	FG	FG	FG	FG	FG	FG
MRSA 12	FG	FG FG	FG	FG	FG	FG	FG	FG

MRSA 13	FG	FG FG	FG	FG	FG	FG	FG	FG
MRSA 14	FG	FG FG	FG	FG	FG	FG	FG	FG
EMRSA m97 271 064 phage group 14	FG	FG FG	FG	FG	FG	FG	FG	FG
EMRSA g96 139 515 phage group 15	FG	FG FG	FG	FG	FG	FG	FG	FG
EMRSA g96 138 744 phage group 16	FG	FG FG	FG	FG	FG	FG	FG	FG

KEY essential oils	
50 % Manuka, 50 % Geranium	1
50 % Tea tree, 50 % Lavender	2
50 % Tea tree, 50 % Geranium	3
50 % Manuka, 50 % Tea tree	4
50 % Manuka, 50 % Lavender	5
50 % Geranium 50 % Lavender	6

FG= Full growth (no area of clearing)

* SD's were for each was zero

(2) The ZOI of 10 strains of staphylococcus against combined essential oils

	Essential oil combinations							
Essential oil	Lemongrass and Lavender				Lemongrass and Tea tree			
Strain	1	2	Mean	SD±	1	2	Mean	SD±
Oxford S aureus NCTC 6571	20	22	21		26	25	25 5	0 71
S aureus NCBC 11882	20	22	21	1 41	12	11	11 5	0 71
S epidermidis NCTC 11047	30	34	32	2.83	46	49	47 5	2 12
S epidermidis NCTC 7944	35	33	34	1.41	35	35	35	0.00
S. saprophyticus NCIMB 8711	28	28	28	0 00	12	13	12 5	0 71
S haemolyticus NCTC 11042	30	33	31 5	2 12	35	33	34	1 41
Strain T1 MSSA	31	33	32	1 41	25	26	25.5	0 71
Strain T4 MSSA	31	26	28.5	3 54	13	16	14 5	2 12
MSSA (4)	35	36	35 5	0 71	42	45	43 5	2 12
MSSA (46)	22	25	23 5	2 12	15	21	18	4 24
MSSA (47)	62	64	63	1 41	30	30	30	0 00
MSSA (48)	35	35	35	0 00	23	24	23 5	0 71
MRSA 11	15	16	15 5	0 71	12	14	13	1 41
MRSA 12	20	18	19	1 41	15	18	16 5	2 12
MRSA 13	17	18	17 5	0 71	20	23	21 5	2 12
MRSA 14	5	6	5 5	0 71	15	15	15	0 00
MRSA 15	31	33	32	1 41	16	18	17	1 41

MRSA 16	32	33	32 5	0 71	30	32	31	1 41
MRSA 17	22	20	21	1 41	25	22	23 5	2 12
MRSA 20	26	22	24	2 83	15	14	14 5	0 71
MRSA 26	32	30	31	1 41	25	22	23 5	2 12
EMRSA m97 271 031 phage group 1	45	47	46	1.41	38	39	38 5	0 71
EMRSA m97 271 038 phage group 2	45	47	46	1 41	19	19	19	0 00
EMRSA j95 922 phage group 3	30	31	30 5	0 71	42	42	42	0 00
EMRSA m97271052 phage group 4	30	25	27 5	3 54	40	40	40	0 00
EMRSA m972 71041 phage group 5	38	42	40	2 83	45	45	45	0 00
EMRSA m97 271 088 phage group 6	30	33	31 5	2 12	45	45	45	0 00
EMRSA m97 271 047 phage group 8	31	31	31	0 00	17	18	17 5	0 71
EMRSA m972 710 40 phage group 9	24	25	24 5	0 71	32	33	32 5	0 71
EMRSA m97 271 032 phage group 10	30	31	30 5	0 71	90	56	73	24 04
EMRSA m97 271 036 phage group 11	25	27	26	1 41	12	15	13 5	2 12
EMRSA m97 271 042 phage group 12	20	19	19 5	0 71	25	24	24 5	0 71
EMRSA m97 271 064 phage group 14	11	9	10	1 41	10	9	9 5	0 71
EMRSA g96 139 515 phage group 15	20	23	21.5	2 12	18	18	18	0 00
EMRSA g96 138 744 phage group 16	15	18	16 5	2 12	20	20	20	0 00
EMRSA g96 136 210 phage group 17	40	40	40	0 00	90	61	75 5	20 51

Essential oil	Essential oil combinations							
	Lemongrass and Manuka				Lemongrass and Geranium			
Strain	1	2	Mean	SD±	1	2	Mean	SD±
Oxford S aureus NCTC 6571	20	22	21	1 41	15	12	13 5	2 12
S. aureus NCBC 11882	8	5	6 5	2 12	8	5	6 5	2 12
S epidermidis NCTC 11047	42	45	43 5	2 12	35	36	35 5	0 71
S epidermidis NCTC 7944	48	48	48	0 00	29	30	29 5	0 71
S saprophyticus NCIMB 8711	34	38	36	2 83	19	18	18.5	0 71
S haemolyticus NCTC 11042	25	26	25.5	0 71	25	21	23	2 83
Strain T1 MSSA	10	11	10 5	0 71	10	10	10	0 00
Strain T4 MSSA	73	79	76	4 24	15	14	14 5	0.71
MSSA (4)	32	33	32.5	0 71	22	22	22	0 00
MSSA (46)	15	15	15	0 00	17	18	17 5	0 71
MSSA (47)	32	32	32	0.00	21	21	21	0 00
MSSA (48)	19	18	18 5	0 71	15	14	14 5.	0 71

MRSA 11	FG	FG	FG	FG	10	11	10 5	0 71
MRSA 12	15	11	13	2 83	10	9	9 5	0 71
MRSA 13	15	13	14	1 41	17	17	17	0 00
MRSA 14	22	24	23	1 41	15	16	15 5	0 71
MRSA 15	15	16	15 5	0 71	19	17	18	1 41
MRSA 16	20	21	20 5	0 71	29	29	29	0 00
MRSA 17	30	30	30	0 00	9	9	9	0 00
MRSA 20	21	24	22 5	2 12	16	18	17	1 41
MRSA 26	25	24	24 5	0 71	25	26	25 5	0 71
EMRSA m97 271 031 phage group 1	52	55	53 5	2 12	38	38	38	0 00
EMRSA m97 271 038 phage group 2	17	17	17	0.00	19	19	19	0 00
EMRSA j95 922 phage group 3	23	23	23	0 00	42	47	44.5	3 54
EMRSA m97271052 phage group 4	25	25	25	0 00	40	42	41	1 41
EMRSA m972 71041 phage group 5	37	37	37	0 00	45	44	44 5	0 71
EMRSA m97 271 088 phage group 6	22	21	21 5	0 71	45	44	44 5	0 71
EMRSA m97 271 047 phage group 8	26	29	27 5	2 12	17	17	17	0 00
EMRSA m972 710 40 phage group 9	25	24	24.5	0 71	32	32	32	0 00
EMRSA m97 271 032 phage group 10	35	34	34 5	0 71	14	15	14 5	0 71
EMRSA m97 271 036 phage group 11	38	36	37	1 41	23	21	22	1.41
EMRSA m97 271 042 phage group 12	28	29	28 5	0 71	25	24	24.5	0 71
EMRSA m97 271 064 phage group 14	13	13	13	0 00	18	18	18	0 00
EMRSA g96 139 515 phage group 15	11	10	10 5	0 71	18	18	18	0 00
EMRSA g96 138 744 phage group 16	30	26	28	2 83	30	31	30 5	0 71
EMRSA g96 136 210 phage group 17	30	32	31	1 41	25	29	27	2 83

ZOI for essential oils and essential oil combinations against all strains

Essential oil	Clearing (range, mm)
Lavender	FG*
Geranium	FG*
Lemongrass	18-86
Manuka	FG*
Tea tree	FG-25 5

FG = Full growth (no area of clearing)

* = Initial tests did not show good results so the effect of the oils on all strains was not pursued

Essential oil combination	50:50 ZOI (range, mm)	75 25 ZOI (range, mm)	25:75 ZOI (range, mm)
Manuka and Geranium	FG*	FG*	FG*
Manuka and Tea tree	FG*	FG*	FG-31.5
Manuka and Lemongrass	FG-76	FG*	FG-72.5
Manuka and Lavender	FG*	FG*	FG*
Tea tree and Lemongrass	9.5-75.5	FG-41	FG-60.5
Tea tree and Lavender	FG*	FG*	FG *
Tea tree and Geranium	FG*	FG-24 5	FG*
Lemongrass and Lavender	5.5-63	11.5-53.5	FG-52.5
Lemongrass and Geranium	6.5-44.5	12-66 5	FG*
Geranium and Lavender	FG*	FG*	FG*

FG = Full growth (no area of clearing)

* = Initial tests did not show good results so the effect of the oils on all strains was not pursued

CONCLUSION

The vapours of essential oils and essential oil combinations that gave the best results (greatest ZOI) were.

50 % Lemongrass and 50 % Tea tree

75 % Lemongrass and 25 % Lavender

75% Lemongrass and 25 % Geranium

- All three combinations were used in further studies
- The vapours of combinations of some essential oils were more effective than when the essential oils were used singly.

5. Estimation of the Minimum inhibitory concentrations (MIC) of all strains against Triclosan (Direct contact)

A stock solution of triclosan was prepared by adding 256 mg of triclosan to 10 ml of Dimethyl sulphoxide. A working solution was then prepared by diluting 1 ml of the stock solution in 9 ml of antibiotic assay broth (AAB). The working solution was then diluted from 2560 μg ml to 0.31 μg ml using AAB. Each dilution of triclosan (1 ml) was then vortex mixed with 19 ml of molten sensitivity test agar (STA) and poured into petri dishes. When set, the dilutions of triclosan in the STA ranged from 128μg ml to 0.01μg ml.

An overnight broth culture (ONBC) of each bacterial strain was diluted 1/100 using AAB. Each strain was then placed onto the surface of the plates using a multi-point inoculator. Each plate was dried for 20 minutes and incubated for 24 hours at 37°C The MIC of each strain was determined as the first plate in the dilution series showing no growth of the organism.

RESULTS

The MIC for triclosan against all strains of staphylococci.

Strain	MIC (Mg ml^{-1})
Oxford S aureus NCTC 6571	0.63
S aureus NCBC 11882	2
S epidermidis NCTC 11047	0.63
S. epidermidis NCTC 7944	2
S. saprophyticus NCIMB 8711	2
S haemolyticus NCTC 11042	1
Strain T1 MSSA	0.5
Strain T4 MSSA	0 25
MSSA (4)	0 5
MSSA (46)	2
MSSA (47)	0.5
MSSA (48)	1
MRSA 11	0.5
MRSA 12	0 5
MRSA 13	2
MRSA 14	0.5
MRSA 15	1
MRSA 16	0.062
MRSA 17	0.25
MRSA 20	1

MRSA 26	2
EMRSA m97 271 031 phage group 1	0.5
EMRSA m97 271 038 phage group 2	0.5
EMRSA j95 922 phage group 3	0 5
EMRSA m97271052 phage group 4	0.062
EMRSA m972 71041 phage group 5	0.062
EMRSA m97 271 088 phage group 6	1
EMRSA m97 271 047 phage group 8	2
EMRSA m972 710 40 phage group 9	1
EMRSA m97 271 032 phage group 10	0.5
EMRSA m97 271 036 phage group 11	0.5
EMRSA m97 271 042 phage group 12	1
EMRSA m97 271 064 phage group 14	0 5
EMRSA g96 139 515 phage group 15	2
EMRSA g96 138 744 phage group 16	1
EMRSA g96 136 210 phage group 17	1

The standard deviation for all was zero.

CONCLUSION

The concentration of triclosan able to inhibit all strains of staphylococci was 2 μg ml^{-1}

6. Estimation of the Minimum inhibitory concentration (MIC) of combined triclosan and essential oils against all staphylococci strains (Direct contact)

Triclosan (4 μg ml) was added to 0.063 % essential oil in equal volumes (concentrations of each were determined in previous experiments). This resulted in a 1/2 dilution of each component (2 μg ml triclosan and 0.031 % essential oil) The combination was then double diluted and 1 ml of each dilution added to 19 ml of molten STA and dispensed into individual petri dishes. Each was allowed to set and dried for 30 minutes.

An overnight broth culture of each bacterial strain was then diluted 1/10 using AAB Each strain was then placed onto the surface of the STA containing each combination of oil and triclosan using

An overnight broth culture of each bacterial strain was then diluted 1/10 using AAB Each strain was then placed onto the surface of the STA containing each combination of oil and triclosan using a multi-point inoculator Each plate was allowed to dry for 20 minutes and incubated for 24 hours at 37°C The MIC of each strain was determined as the first plate within the dilution series containing no growth of the organism

RESULTS

The MIC of combined essential oils and triclosan against all strains of staphylococci

Strain	Manuka and Geranium (1)	Manuka and Tea tree (2)	Manuka and Lemongrass (3)	Tea tree and Lavender (4)	Lemongrass and Geranium (5)
Oxford S aureus NCTC 6571	F	E	F	D	D
S aureus NCBC 11882	F	D	D	D	D
S. epidermidis NCTC 11047	F	E	E	D	E
S epidermidis NCTC 7944	F	D	D	D	D
S saprophyticus NCIMB 8711	F	D	F	D	C
S. haemolyticus NCTC 11042	F	D	F	D	E
Strain T1 MSSA	F	F	E	D	E
Strain T4 MSSA	F	E	E	D	E
MSSA (4)	F	E	F	D	E
MSSA (46)	F	D	D	F	D
MSSA (47)	F	F	F	D	E
MSSA (48)	F	E	E	F	E
MRSA 11	F	D	D	D	E
MRSA 12	F	E	E	D	E
MRSA 13	F	D	D	F	C
MRSA 14	F	F	E	D	E
MRSA 15	F	F	E	D	E
MRSA 16	F	F	E	D	E
MRSA 17	F	F	D	D	E
MRSA 20	F	F	F	F	E
MRSA 26	F	D	D	D	E
EMRSA m97 271 031 phage group 1	F	F	E	F	E
EMRSA m97 271 038 phage group 2	F	E	F	D	E
EMRSA j95 922 phage group 3	F	F	F	F	E
EMRSA m97271052 phage group 4	F	F	E	F	E
EMRSA m972 71041 phage group 5	F	F	F	F	E
EMRSA m97 271 088 phage	F	F	E	F	E

10					
EMRSA m97 271 036 phage group 11	F	E	E	F	E
EMRSA m97 271 042 phage group 12	F	E	E	F	E
EMRSA m97 271 064 phage group 14	G	F	E	F	E
EMRSA g96 139 515 phage group 15	F	D	D	D	D
EMRSA g96 138 744 phage group 16	F	E	E	F	E
EMRSA g96 136 210 phage group 17	F	E	E	F	E

Key for oils 1,2,3			
	Oil		Triclosan
A	0 031	+	2
B	0 016	+	1
C	0 008	+	0.5
D	0 004	+	0 25
E	0 002	+	0 125
F	0 001	+	0 0625
G	0 0005	+	0.03125

Key for oils 4 and 5			
	Oil		Triclosan
A	0 125	+	2
B	0 063	+	1
C	0 031	+	0 5
D	0 016	+	0 25
E	0 008	+	0 125
F	0.004	+	0 0625
G	0 002	+	0 03125

CONCLUSION

- A lower concentration of combined triclosan and essential oils were more effective at inhibiting growth of all strains compared to when used singly

7 Assessment of the vapours of triclosan against all strains of staphylococcus (Vapour phase)

A stock solution of triclosan was prepared by adding 256 mg of triclosan to 10 ml of Dimethyl sulphoxide A working solution was then prepared by diluting 1 ml of the stock solution in 9 ml of antibiotic assay broth (AAB) The working solution (20µl) was then placed onto 6 mm filter paper discs and placed into the lid of petri dishes

An ONBC of each bacterial strain was diluted 1/100 and swabbed onto the surface of STA The lids containing the discs were placed onto the petri dishes and the plates incubated for 24 hours at 37 °C The ZOI of each strain was determined by measuring the area of bacterial cleaning (diameter, mm)

RESULTS

The ZOI of all strains against triclosan vapours

Strain	1	2	Average	SD±
Oxford S. aureus NCTC 6571	50	52	51	1 41
S. aureus NCBC 11882	47	49	48	1.41
S. epidermidis NCTC 11047	50	51	50.5	0.71
S. epidermidis NCTC 7944	50	51	50 5	0.71
S. saprophyticus NCIMB 8711	33	42	37 5	6.36
S haemolyticus NCTC 11042	48	44	46	2.83
Strain T1 MSSA	46	45	45.5	0.71
Strain T4 MSSA	50	52	51	1.41
MSSA (4)	50	52	51	1.41
MSSA (46)	45	45	45	0 00
MSSA (47)	52	51	51.5	0.71
MSSA (48)	49	48	48.5	0 71
MRSA 11	43	43	43	0 00
MRSA 12	45	44	44.5	0.71
MRSA 13	47	47	47	0.00
MRSA 14	55	55	55	0.00
MRSA 15	50	50	50	0.00
MRSA 16	55	56	55.5	0 71
MRSA 17	31	33	32	1.41
MRSA 20	55	55	55	0.00
MRSA 26	40	40	40	0 00
EMRSA m97 271 031 phage group 1	42	40	41	1.41
EMRSA m97 271 038 phage group 2	57	57	57	0 00
EMRSA j95 922 phage group 3	55	53	54	1.41
EMRSA m97271052 phage group 4	50	50	50	0
EMRSA m972 71041 phage group 5	55	55	55	0
EMRSA m97 271 088 phage group 6	52	52	52	0
EMRSA m97 271 047 phage group 8	52	52	52	0
EMRSA m972 710 40 phage group 9	55	55	55	0
EMRSA m97 271 032 phage group 10	49	49	49	0
EMRSA m97 271 036 phage group 11	52	52	52	0
EMRSA m97 271 042 phage group 12	45	45	45	0
EMRSA m97 271 064 phage group 14	35	35	35	0
EMRSA g96 139 515 phage group 15	47	47	47	0
EMRSA g96 138 744 phage group 16	50	50	50	0
EMRSA g96 136 210 phage group 17	47	47	47	0

SD for all is zero

CONCLUSION

The vapours of triclosan were effective at inhibiting growth of all staphylococcal strains

8 Assessment of the vapours of triclosan and essential oils against all strains of staphylococcus (Vapour phase)

Triclosan (2560µg ml^{-1}) was added in equal volumes to essential oil (100%) (The concentrations of essential oils were determined in previous experiments) This resulted in a 1/2 dilution of each component (1280µg ml triclosan and 50% essential oil) The combination was then added to a 6 mm filter paper disc and placed on the lid of a petri dish

An ONBC of each bacterial strain was diluted 1/100 and swabbed onto the surface of STA The lids containing the discs were placed onto the petri dishes and the plates incubated for 24 hours at 37 °C

The STA of the petri dishes was then surface swabbed with a 1/100 dilution of the ONBC of 10 selected staphylococci strains and the petri dish placed onto the petri dish Plates were incubated for 24 hours at 37 °C The ZOI of each strain was determined by measuring the area of bacterial clearing (diameter, mm)

Note 10 strains were initially screened to assess if they had any effect on growth

RESULTS

The ZOI of 10 staphylococcal strains against the vapours of combined triclosan and essential oils

Strain	Tea tree and Lemongrass	Lemongrass and Lavender	Lemongrass and Geranium
Oxford S aureus NCTC 6571	FG	19	FG
S aureus NCBC 11882	30	FG	FG
S epidermidis NCTC 11047	35	FG	FG
S haemolyticus NCTC 11042	FG	FG	FG
Strain T4 MSSA	15	FG	FG
MRSA 12	12	FG	20
MRSA 13	29	FG	10

MRSA 14	FG	FG	FG
EMRSA m97 27 I 064 phage group 14	FG	FG	FG
EMRSA g96 139 515 phage group 15	FG	FG	FG
EMRSA g96 138 744 phage group 16	FG	FG	FG

FG= Full growth (no area of clearing)

Standard deviations for each were zero

CONCLUSION

- When essential oils and triclosan were combined, the effect of the vapours on the ZOI was less effective than when used singly This indicates that when combined an antagonistic effect between the triclosan and oils were occurring

9 Assessment of essential oil and encapsulated essential oil combinations on the growth of EMRSA 16 when incorporated into calcium alginate for use as a wound dressing

Essential oil combinations (100 µl, combinations previously determined) were added to 20 ml of calcium, alginate and then placed into the lid of a petri dish An ONBC of EMRSA 16 was diluted 1/100 and swabbed onto the surface of STA The lid was placed on the petri dish and the plates incubated at 37°C for 24 hours

RESULTS

ZOI of EMRSA 16 against combinations of essential and encapsulated essential oils in calcium alginate

	Essential oil	Encapsulated essential oil
Tea tree and Lemongrass 50 50	No growth	No growth
Lemongrass and Lavender 75 25	No growth	No growth
Lemongrass and geranium 75 25	No growth	No growth

CONCLUSION

Both the essential oils and encapsulated essential oils prohibited growth of strain EMRSA over 24 hours.

10 Assessment of essential oil and encapsulated essential oil combinations on the growth of EMRSA 16 when incorporated into gauze for use as a wound dressing

Essential oil combinations (100 µl, combinations previously determined) were added to 10 ml of water and then used to soak a 15cm^2 piece of added to gauze An ONBC of EMRSA 16 was diluted 1/100 and swabbed onto the surface of STA The gauze dressing was then placed over the top of the petri dish and the lid replaced (the gauze and the STA were not touching). The plates were then incubated for 24 hours at 37°C and the ZOI measured

RESULTS

ZOI of EMRSA 16 against combinations of essential and encapsulated essential oils contained in gauze

	Essential oil	Encapsulated essential oil
Tea tree and Lemongrass 50:50	No growth	No growth
Lemongrass and Lavender 75:25	No growth	No growth
Lemongrass and geranium 75.25	No growth	No growth

CONCLUSION

Both the essential oils and encapsulated essential oils prohibited growth of strain EMRSA over 24 hours.

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CLAIMS

1 A composition for use as a medicament wherein the composition comprises at least one essential oil and a fungal cell or fungal cell fragment wherein molecules of the at least one essential oil are encapsulated or partially encapsulated by the fungal cell or fungal cell fragment.

2. A composition comprising at least one essential oil and at least one biocidal compound.

3 A composition as claimed in claim 2 wherein the biocidal compound comprises a fungicide, an antibiotic and/or a bactericide

4 A composition as claimed in claim 3 wherein the biocidal compound comprises one or more selected from the group comprising triclosan, mupirocin, chlorhexidine, povidone iodine and silver sulphadiazine.

5. A composition comprising an essential oil, a biocidal compound and a fungal cell or fungal cell fragment, wherein molecules of at least one of the essential oil or biocidal compound are encapsulated or partially encapsulated by the fungal cell or fungal cell fragment

6. A composition comprising two or more essential oils and a fungal cell or fungal cell fragment wherein molecules of at least one essential oil is encapsulated or partially encapsulated by the fungal cell or fungal cell fragment.

7. A therapeutic formulation comprising a composition as claimed in any one of the previous claims.

8 The use of a composition for the manufacture of a medicament for the treatment of microbial infection, the composition comprising at least one essential oil and a fungal cell or fungal cell fragment, wherein molecules of the essential oil are encapsulated or partially encapsulated by the fungal cell or fungal cell fragment

9 The use of a composition as claimed in claim 8 wherein the use is for the treatment of Staphylococcus infection.

10 The use of a composition as claimed in claim 9 wherein the use is for the treatment of *S. aureus*, *S epidermidis*, *S. saprophyticus*, *S. haemolyticus*, Methicillin sensitive *S aureus*

(MSSA), Methicillin resistant *S. aureus* (MRSA) and/or Epidemic methicillin resistant *S. aureus* (EMRSA).

11. The use of a composition as claimed in claim 10 wherein the use is for the treatment of MRSA

12. A method of preventing a microbial infection in a subject comprising administering to a subject a composition as claimed in any one of claims 1 to 7.

13 A composition comprising

a) a first essential oil comprising Manuka and a second essential oil comprising one or more essential oil selected from the group comprising Geranium, Lavender, Lemongrass, and Tea tree; or

b) a first essential oil comprising Geranium and a second essential oil comprising one or more essential oil selected from the group comprising Manuka, Lavender, Lemongrass, and Tea tree; or

c) a first essential oil comprising Lemongrass and a second essential oil comprising one or more essential oil selected from the group comprising Geranium, Lavender, Manuka, and Tea tree; or

d) a first essential oil comprising Lavender and a second essential oil comprising one or more essential oil selected from the group comprising Geranium, Manuka, Lemongrass, and Tea tree; or

e) a first essential oil comprising Tea tree and a second essential oil comprising one or more essential oil selected from the group comprising Geranium, Lavender, Lemongrass, and Manuka.

14 A wound dressing comprising a carrier and a composition or formulation as claimed in any one of claims 1 – 7 and 13.

Application No:	GB0321130.7	**Examiner:**	Dr Rowena Dinham
Claims searched:	1, 6 & 8-11; and 7, 12 & 14 (in part)	**Date of search:**	14 December 2004

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X,Y	X: 1, 6 & 7; Y: 8-12 & 14	EP 0242135 A3 (AD2 Ltd) See especially page 2 line 28-page 3 line 13, page 4 line 9-14, and Examples
X,Y	X: 1, 6 & 7; Y: 8-12 & 14	US5798525 A (Hobson) See entire document especially column 1 line 44-column 2 line 35 and Examples
Y	All	EP 0085805 A1 (Dunlop Ltd) See especially page 1 line 32- page 2 line 10, page 2 line 28- page 3 line 6 and Examples
Y	All	WO 00/69440 A3 (Micap Ltd) See especially page 2 line 26-27 and page 4 line 7-17
Y	All	WO 2003/028451 A3 (State of Israel, Ministry of Agriculture) See especially page 5 line 2-8, page 6 line 21-23 and Examples
Y	All	US5635184 A (Camano) See entire document, especially column 1 line 3-50
Y	All	US4966754 A (Purohit) See entire document, especially column 1 line 37-65
Y	1, 6-8 & 14	US 6680074 B1 (Morice) See entire document, especially column 1 line 4-49 and Examples
Y	1, 6-8 & 14	J Essent Oil Res; Vol 13, pp 387-392 (2001). Horne et al. "Antimicrobial effects of essential oils on Streptococcus pneumoniae" See entire document, especially Results, Discussion and Tables I-III

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

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Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{W} :

Worldwide search of patent documents classified in the following areas of the IPC^{07}

A61K

The following online and other databases have been used in the preparation of this search report

WPI, EPODOC, JAPIO, MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS